

Regulation of legume nodulation by acidic growth conditions

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Legumes represent some of the most important crop species worldwide. They are able to form novel root organs known as nodules, within which biological nitrogen fixation is facilitated through a symbiotic interaction with soil-dwelling bacteria called rhizobia. This provides legumes with a distinct advantage over other plant species, as nitrogen is a key factor for growth and development. Nodule formation is tightly regulated by the plant and can be inhibited by a number of external factors, such as soil pH. This is of significant agricultural and economic importance as much of global legume crops are grown on low pH soils. Despite this, the precise mechanism by which low pH conditions inhibits nodule development remains poorly characterized.

The Development and Regulation of Legume Nodulation

Many legumes have evolved to establish a symbiosis with nitrogen-fixing soil-bacteria collectively known as rhizobia (including the genera *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium*). Rhizobia invade the roots of compatible legume plants leading to the development of specialized root structures called nodules.¹⁻⁴ Within these nodules, the nitrogenase enzyme complex of rhizobia reduces atmospheric nitrogen, an unusable form of nitrogen for plants, into ammonia, which the plant utilizes for growth and development.^{5,6}

Nodule formation and nitrogen fixation are energy expensive and are therefore tightly regulated to ensure a balance between nitrogen acquisition and energy expenditure. One internal control mechanism of the plant is the Autoregulation of Nodulation (AON), a regulatory process acting via long distance signaling.^{1-3,7-9} AON is initiated during early nodule development by the production of a rhizobia-induced signal in the root, which is mobilised to the shoot.¹⁰ In soybean, *GmRIC1* and *GmRIC2* of the CLAVATA3/ESR-related (CLE) family of peptides, have

been identified as strong candidates for this signal.¹¹⁻¹⁴ Similar peptides involved in AON have also been identified in *Lotus japonicus* and *Medicago truncatula*.^{15,16} Upon perception of the rhizobia-induced signal in the shoot, a novel factor called the Shoot-Derived Inhibitor (SDI) is produced and is predicted to be transported down to the root where it acts to inhibit continued nodule development.^{17,18}

External factors can also regulate legume nodule numbers. For example, the plant hormone ethylene is a strong inhibitor of nodulation and is produced following stress, enabling the plant to reduce nodule production when growing conditions are sub-optimal.¹⁹ Many nitrogenous compounds are also strong inhibitors of nodule formation. Legume plants have evolved a mechanism to detect nitrogenous compounds in the soil, such as nitrate and ammonium, which enables them to reduce nodule development when ample sources of nitrogen are already available.^{2,9} The presence of these nitrogenous compounds in the soil triggers the production of a CLE peptide signal, called GmNIC1 in soybean, which is highly similar to GmRIC1/2 in AON.^{2,14} However, this nitrogen-sensing process acts locally in the root, as opposed to AON, which acts systemically through the shoot.¹⁴

Factors such as soil composition, water content, temperature and pH can also influence plant and rhizobia growth,^{20,21} and nodule establishment.^{22,23} Indeed, soil acidity alone is responsible for significant losses in global legume production, resulting from impaired plant and rhizobia growth, in addition to decreased nodule development and nitrogen fixation. The following review highlights the effects low soil pH has on this critical legume-rhizobia symbiosis.

Plant Growth in Low pH Soil

Over 1.5 Gha of the world's soils are acidic (pH < 5.5; Fig. 1), with up to 40% of arable land affected by soil acidity.^{24,25} Low soil pH is often caused by poor nutrient cycling, soil leaching and the acidifying effects of nitrogen fertiliser.^{25,26} They are responsible for yield losses of 50% or more in wheat and barley crops, as well as legume crops, such as lentil, bean and pea.²⁷⁻³⁰ Interestingly, legumes tend to acidify soil to a greater extent than many other species.^{31,32}

Elevated levels of toxic anions in low pH soil have compounding effects on plant growth. Plant apoplasts are weak cation

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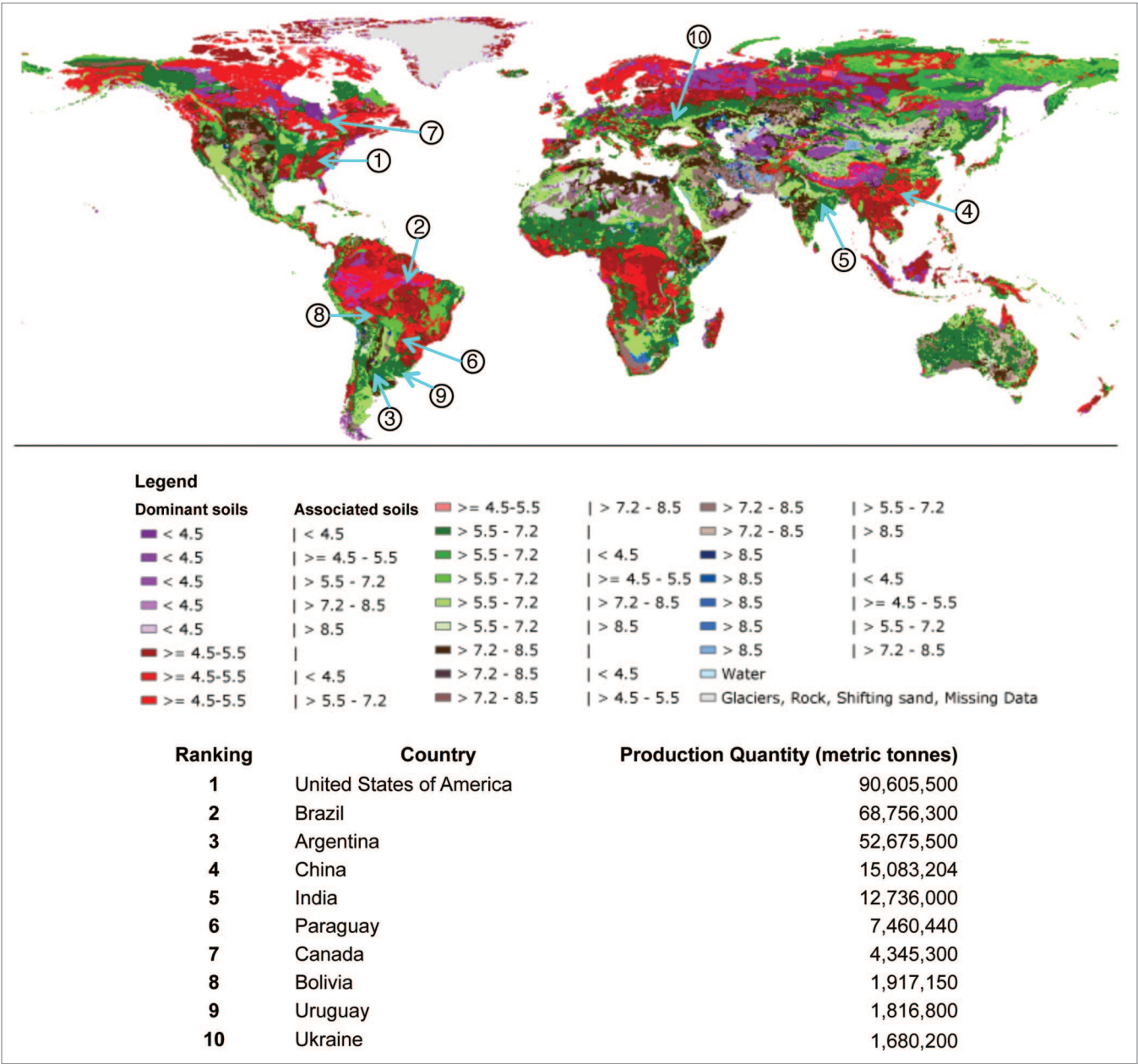


Figure 1. World soil pH distribution and the top 10 global soybean producers (quantity; metric tonnes; FAOSTAT, 2010). As is the case with many legume crops, soybean production is predominantly located in regions of low pH soil. Map adapted from: FAO-UNESCO Soil Maps of the World, 2007.

exchangers that require Ca^{2+} and Mg^{2+} loading to increase the uptake of cations such as Mg^{2+} , Zn^{2+} , Mn^{2+} and Ca^{2+} .²⁰ High H^+ and Mn^{2+} concentration in low pH soil outcompetes Ca^{2+} and Mg^{2+} loading and also creates a steep concentration gradient between the rhizodermal cell cytoplasm and the rhizosphere.³³ This favors anion uptake (through the apoplast) over the uptake of Mg^{2+} , Ca^{2+} and K^+ .³³

High Al^{3+} concentration is identified as the main limiting factor on plant viability in 67% of all low pH soils.³⁴ Aluminum hydrolyzes in low pH solutions ($\text{pH} < 5$) to form the toxic trivalent form, Al^{3+} , which is prevalent in acid soils.³⁵ Root apices are the primary sites of Al^{3+} accumulation and therefore suffer more

physical damage than mature root regions.³⁶ Al^{3+} toxicity inhibits root cell division and elongation³⁶ and decreases macronutrient availability and retention by binding pores in the soil. Al^{3+} also inhibits cation uptake by outcompeting Ca^{2+} and Mg^{2+} loading in the apoplast.³⁷

Several adaptation mechanisms enable plants to survive in low pH soils. The impacts of low nutrient availability are reduced by slowing their growth rate, lowering their nutrient use efficiency (the amount dry matter produced per unit nutrient)³⁸ and lowering their internal nutrient demand and recirculation of mineral nutrients.²⁰ Adaptation to toxic ions is achieved through

restricting influx at the plasma membrane and at sensitive zones in the root, or by compartmentalisation of toxic ions in cell walls.^{39,40}

Rhizobia Growth in Low pH Soil

Rhizobia growth, survival and abundance, in addition to their competitiveness in nodulation, are highly influenced by soil pH.⁴¹⁻⁴⁵ Increased H^+ concentration and increased solubility of the toxic metal ions Al^{3+} , Cu^{2+} and Mn^{2+} are the primary causes of inter-cellular pH instability leading to growth inhibition in low pH soils.^{46,47} Acid tolerant strains of many rhizobia species have been isolated,^{43,47} with pH tolerance often being facilitated by proton exclusion,⁴⁸ increased cytoplasmic buffering⁴⁹ and acid-shock response mechanisms.⁴⁶

Strain-specific symbiotic potency varies at different soil pH levels, where strains that are outcompeted under normal pH conditions ($pH > 5.5$) become dominant under low pH ($pH < 5.5$) conditions.^{50,51} *Bradyrhizobium* sp are generally more acid-tolerant than most *Rhizobium* sp.^{41,52} Although few rhizobia thrive at $pH < 5$,^{47,53} certain strains of *R. tropici* and *R. loti* are highly acid-tolerant.^{28,47,54,55}

Inhibition of Nodulation by Low pH Conditions

Legumes species differ in their nodulation and growth response to acidic soil.^{22,24,56} Generally, nodule formation is more sensitive to soil acidity than other aspects of plant growth.²² Species, such as *M. sativa*, are highly sensitive to acidic growth conditions, whereas others, including *Lotus tenuis*, are more tolerant.⁴² In addition, a number of varieties isolated from the highly-acidic Brazilian Cerrado and Caatinga biomes exhibit acid-tolerant nodulation, including certain species of *Lupinus* and *Mimosa*.^{57,58}

In low pH soil, nodule formation has been reported to be reduced by $> 90\%$ and nodule dry weight by $> 50\%$ in species such as soybean, pea, cowpea, *Medicago* and Lucerne, with both determinate- and indeterminate-nodule forming species affected.^{22,59-62} In bean, low soil pH is reported to reduce the number, ultrastructure and weight of nodules, in addition to the nitrogenase activity.³⁰ Indeed, nitrogen fixation is typically reported to be reduced in acidic soil, with both temperate and tropical species being affected.^{24,63} Molybdenum deficiency in low pH soil²⁰ further hinders nitrogen fixation, as it is a key component of the nitrogenase enzyme complex.⁶⁴ The loss of nitrogen acquisition is often overcome by fertilisation, as is reflected by the fact that 75% of the 3.6 million tonnes of nitrogen fertiliser used worldwide each year are applied in major soybean production regions, where low pH soils persist (International Fertilizer Industry Association; www.fertilizer.org; Fig. 1).

Low pH conditions disrupt the signal exchange between the host plant and microsymbiont.⁵⁰ Reduced plant flavonoid secretion⁵⁰ decreases rhizobia *Nod* gene induction and restricts NF and Nod metabolite excretion.^{65,66} The reduction in NF signaling results in the failure of downstream events, such as root hair deformation and curling.^{67,68} Low pH conditions have also been shown to affect rhizobia attachment to root hairs^{45,69} and root colonisation,⁷⁰ leading to reduced nodule formation. Furthermore, low soil

pH also significantly reduces processes occurring downstream of, or in parallel to, root-hair infection. This includes the expression of early nodulation genes,⁷¹ initial cell division events and primordia establishment. However, the precise mechanisms resulting in these reductions remain unclear.⁶⁰ The recent identification of a systemic root-to-shoot-to-root pathway acting to regulate nodulation in response to acidic soil conditions provides a new layer to the complexity of this regulatory framework.⁷¹

Improving Nodulation Under Low pH Soil Conditions

Conventional methods to improve nodulation in low pH soils have addressed plant or rhizobia growth individually. The simplest approach to resolve low soil pH is to raise it to a more suitable level. Liming is a common means of raising soil pH; applying $CaCO_3$ to bind excess H^+ while at the same time releasing Ca^{2+} which can be used as a nutrient.⁷² However, poor mixing of $CaCO_3$ with soil⁷³ and over-liming can reduce crop yield,⁷³ meaning liming is often ineffective.

Plants have also evolved mechanisms to help raise the pH of the soil immediately surrounding their roots. Rhizosphere pH can be modified through root secretion of the alkaline anions OH^- , HCO_3^- ⁷⁴⁻⁷⁵ and metal ion chelators.⁷⁶ Aluminum-tolerance in cereals is well characterized and involves Al -exclusion through the excretion of mixtures of the organic acids citrate, malate and oxalate to chelate Al^{3+} ions and detoxify the rhizosphere.^{77,78}

External genistein (a soybean isoflavone) and purified NF application partially restores nodulation by improving plant-rhizobia signaling and root hair infection.^{67,79} However, this is expensive and not viable for agriculture. Other approaches, including applying acid-tolerant strains of rhizobia, have also been shown to improve symbiotic performance.^{41,53,80-83} To date, the most effective means to improve nodulation under low soil pH conditions is to use acid-tolerant legume cultivars.⁸⁴⁻⁸⁸ Some reports have concluded that proper nodulation can be achieved in acidic growth conditions as long as one of the symbionts is acid-tolerant.⁴⁵ However, poor nodulation can occur even in the presence of a healthy rhizobia population,^{28,47} and in many cases the host legume variety is key to establishing the symbiosis in a low pH environment. Nevertheless, with all of these approaches, nodulation is typically only slightly improved. This can often be attributed to an indirect result of improved plant and rhizobia growth, rather than acid-tolerant nodulation.^{23,24,85,87} However, it also indicates that regulatory components, aside from direct, local pH-induced inhibitory factors, are involved in suppressing nodulation in acidic soil.

Recent Advances in Discovering the Molecular Mechanisms of Acid-Regulation of Nodulation

Though highly prevalent and economically significant, the molecular mechanisms inhibiting legume nodulation by low soil pH have been poorly defined. Recently, Lin et al. (2012) demonstrated, through split-root and grafting studies using an AON-deficient mutant line of soybean, that a systemic, shoot-controlled and GmNARK-dependent mechanism was key to facilitating the inhibitory effect of low soil pH on legume nodulation. Inhibition

was induced early in nodule ontogeny, between 12–96 h post-inoculation with *Bradyrhizobium japonicum*.⁷¹ Furthermore, the transcript abundance of known early nodulation genes was downregulated in response to acid stress, including being systemically downregulated in split root plants.⁷¹ This breakthrough in identifying one of the mechanisms responsible for low soil pH inhibition of legume nodulation has helped to establish and highlight the complexity of this regulatory process. Indeed, it suggests that soil acidity not only effects the growth and development of rhizobia bacteria and legume plants, but also acts to directly inhibit the formation of nodule structures in both a local^{22,60,70} and systemic⁷¹ manner.

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Conclusions

Low pH nodulation studies have largely been based on observing symptomatic effects. Further characterization using more direct molecular genetic approaches are now required to establish a more complete understanding of the mechanisms of low pH inhibition of nodulation. Findings will enable more targeted and practical approaches that could help to reduce this widespread agricultural problem.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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